Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 5 (3): 274-280, 2011
ISSN 1819-3455 / DOI: 10.3923/rjmp.2011.274.280

Peltophorum pterocarpum (DC.) Baker ex. K. Heyne Flowers: Antimicrobial and Antioxidant Efficacies

[1]S.C. Jain, [1]B. Pancholi and [2]R. Jain
[1]Medicinal Plants and Biotechnology Laboratory, Department of Botany,
[2]Department of Chemistry, University of Rajasthan, Jaipur-302004, India

Corresponding Author: Satish C. Jain, Medicinal Plants and Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302004, India

ABSTRACT

Peltophorum pterocarpum, a large tree of wide occurrence, posses varied medicinal properties. In the present study, its flowers were successively extracted with pet. ether, dichloromethane, ethyl acetate and methanol and screened for their potential antimicrobial and antioxidant efficacies. In antibacterial screening, ethyl acetate fraction exhibited maximum activity against *S. aureus* and *E. aerogenes* (IZ 16.00±0.57 and 15.33±0.32 mm, respectively), while dichloromethane fraction was found active against *R. planticola* and *E. aerogenes* (IZ 15.66±1.19, 14.66±0.66 mm, respectively). In antifungal screening, ethyl acetate fraction showed appreciable activity against *T. rubrum* and *P. crysogenum* (IZ 15.00±0.00 mm). The 6.5 μg mL^{-1}) as compared to the standard (quercetin). From the results it is evident that the flowers of *P. pterocarpum*. However, in antioxidant screening, methanol fraction showed highest activity (RC_{50} possess very potent antimicrobial and antioxidant efficacies.

Key words: *Peltophorum pterocarpum*, quercetin, antimicrobial, antioxidant efficacies

INTRODUCTION

Peltophorum pterocarpum (DC.) Baker ex. K. Heyne (Vern. name: Peela Gulmohar; Fam. Caesalpiniaceae) is a deciduous tree growing up to 25-25 m. The bark of tree is used in dysentery, for gargles, tooth powders and externally in eye diseases, muscular pains and sores as lotions (Deshaprabhu, 1966). The bark also gives a dye of a yellow color. From its flowers crystalline compound, peltophorin which was later called as berginin, β-sitosterol, lupeol and a flavonoid glycoside naringenin-7-glucoside (Rao, 1965; Joshi and Kamat, 1969; Rahman *et al*., 1969; Varshney and Dubey, 1969) have been isolated. Antimicrobial activity of benzene and methanol fractions of flowers has been conducted against several Gram-ve and Gram+ve bacteria, where significant results have been demonstrated (Sethuraman *et al*., 1984; Duraipandiyan *et al*., 2006). Likewise, whole plant extracts have been tested against other microbes (Ali *et al*., 2001; Rahman *et al*., 2007; Satish *et al*., 2007, 2008; Voravuthikunchai and Mitchell, 2008). However, in the present study antimicrobial activity against some select bacteria and fungi including its antioxidant activity has been carried out.

MATERIALS AND METHODS

Plant material: During the course of studies, authenticated sample of *Peltophorum pterocarpum* (DC) Baker ex K. Heyne (Caesalpineaceae) were procured from Campus of University of Rajasthan,

Jaipur and others in the month of July-September, 2008-09. Their voucher specimens were deposited in the Herbarium, Botany Department, University of Rajasthan, Jaipur.

Preparation of test extracts: Flowers were shade-dried, crushed and Soxhlet extracted in succsession with pet. ether (A), dichloromethane (B), ethyl acetate (C) and methanol (D) for 72 h. These extracts were filtered, concentrated to dryness *in vacuo* and stored at appropriate temperature for further studies. The yield (%) of each fraction (A-D) was found to 2.45, 0.80, 0.78 and 6.50, respectively.

Antimicrobial efficacy

Bioassays: Pure cultures of test bacteria, *Bacillus subtilis* (MTCC 441), *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (ATCC 443), *Pseudomonas aeruginosa* (ATCC 741), *Raoultella planticola* (MTCC 530) and *Staphylococcus aureus* (ATCC 740) were obtained from the IMTECH, Chandigarh, India. These cultures were grown and maintained on Nutrient broth medium (NB) at 27°C for 48 h. In fungus, *Aspergillus flavus* (16870) *A. niger* (ATCC 322), *Candida albicans* (ATCC 4718), *Penicillium crysogenum* (ATCC 5476) and *Tricophyton rubrum* (ATCC 2327) obtained from IARI, New Delhi, India, were cultured on Sabouraud's dextrose agar (SDA) at 37°C for 48 h. Antimicrobial tests were performed by agar well diffusion method (Boyanava *et al*., 2005). Inoculum of each bacterial in nutrient broth and fungi on SDA broth were prepared at 25°C for the period of 36 h for the adjustment of appropriate concentration (10^6-10^7 cfu mL^{-1}). Twenty microlitter of bacterial inoculum and 80 μL of fungal inoculum are inoculated in Müller-Hinton and SDA, respectively. Four milligram extract was delivered to each well. To ensure diffusion of sample into agar, plates were incubated at 4°C for 1 h, which were then incubated at 37°C for bacteria and 25°C in fungi for appropriate time periods under aerobic conditions. The diameter of the inhibition zone around each hole was measured and recorded (Inhibition zone recorder, HiMedia, India). Three replicates were used and the average value was statistically analyzed. Gentamycin (10 mcg $disc^{-1}$) and ketonocozole (10 mcg $disc^{-1}$) were used as positive controls.

Total phenolic contents: The total phenolic contents were determined using Folin-Ciocalteau reagent (Maehly and Chance, 1954). Optical Density (OD) was measured at 750 nm (Pharmaspec UV- Vis spectrophotometer by Shimadzu). A standard calibration curve of gallic acid (10-500 mg L^{-1}) was prepared and total phenolics in the extract were expressed in mg of gallic acid equivalents (mg GAE/100 g of extract). All determinations were carried out in triplicate and statistically analyzed.

Total flavonoids contents: Total flavonoids were estimated by $AlCl_3$ spectrophotometric method (Zhishen *et al*., 1999). Standard curve of quercetin (10-100 mg mL^{-1}) was prepared. The total flavonoids were expressed as mg of quercetin equivalents (mg QE/g) of extract and statistically analyzed.

Antioxidant activity

Free radical scavenging activity by 2, 2-diphenyl-1-picryl-hydrazyl: The effect on DPPH radical was determined using the method by Fogliano *et al*. (1999). Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The capability to scavenge the DPPH radical was calculated using following equation:

$$\text{DPPH scavenging effect (\% Inhibition)} = \left[\left(\frac{A_0 - A_1}{A_0}\right) \times 100\right]$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of given extract.

Total reduction capability by FRAP method: Ferric ion Reducing Antioxidant Potential (FRAP) of extracts was determined according to Yen and Chen (1995). The absorbance was measured at 700 nm using a UV-Vis spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. Standard calibration curve of ascorbic acid was prepared using 10-500 mg L^{-1} and total antioxidant potentials were calculated in mg of ascorbic acid equivalents (mg AAE/g of extract). All determinations were carried out in triplicate and statistically analyzed.

Metal chelating activity on ferrous ions: Ferrous ion (Fe^{+2}) chelation was estimated by the ferrozine assay (Dinis *et al.*, 1994). Optical density was measured at 562 nm. All tests were run in triplicate and averaged. The % inhibition of ferrozine Fe^{+2} complex formations was calculated as follows:

$$\text{Inhibition \%} = \left[\left(\frac{A_0 - A_1}{A_0}\right) \times 100\right]$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of given extract. The control contained $FeCl_2$ and ferrozine complex molecules.

RESULTS

Total yield of crude extracts was found to be variable. The extracts were prepared on the basis of polarity of different secondary metabolites. All the four extracts (pet. ether, dichloromethane, ethyl acetate and methanol) demonstrated appreciable activity against most of the test bacteria and fungi (Table 1). In antibacterial screening, the potential activity 16.00±0.57 and 15.33±0.32 mm (with MIC 125 μg mL^{-1} in both cases) was demonstrated by ethyl acetate extract at 4 mg/well concentration against *R. planticola* and *S. aureus*, respectively. Likewise, against fungi, pronounced activity 14.33±0.33, 15.00±0.00 and 15.00±0.00 mm was recorded in same extract against *C. albicans*, *P. crysogenum* and *T. rubrum* (MIC and 62.5 μg mL^{-1} in *P. crysogenum* and *T. rubrum*, respectively).

On investigation of total phenolics and flavonoids, methanol extract showed maximum levels of phenolics (74.66±0.32 mg GAE/100 g) while flavonoids were higher in ethyl acetate extract (29.61±0.43 mg QE/g) (Table 2).

It is evident that methanol extract was the most active among all the four extract. In DPPH method, % inhibition of methanol and ethyl acetate extracts were 4 and 6.5 μg mL^{-1}, respectively (Table 3). Methanol extracts exhibited antioxidant activity in concentration dependent manner viz., 80 μg mL^{-1} showed 84.43% inhibition as compared to 94.71% as compared to quercetin. In FRAP method, maximum absorbance was demonstrated by methanol extract where 1000 μg concentration

Table 1: Antibacterial activity of *P. pterocarpum* flowers

	[a]Type of extract			
Microorganisms	Pet ether	Dichloromethane	Ethyl acetate	Methanol
B. subtilis				
IZ[b]	12.66±0.66	14.00±0.33	15.33±0.32	12.33±0.57
MIC[c]	250	62.5	250	125
AI[d]	0.57	0.75	0.63	0.56
E. aerogens				
IZ	11.00±0.57	14.66±0.66	14.66±0.88	13.00±1.00
MIC	125	62.5	125	1000
AI	0.78	1.04	1.04	0.92
P. aruginosa				
IZ	12.00±0.57	13.00±0.57	13.00±0.57	14.66±0.57
MIC	250	125	1000	62.5
AI	0.60	0.65	0.67	0.66
R. planticola				
IZ	14.33±0.33	15.66±0.32	15.33±0.32	12.33±0.32
MIC	500	125	125	62.5
AI	0.63	0.71	0.69	0.56
S. areus				
IZ	11.66±0.66	14.00±1.35	16.00±0.57	13.00±0.57
MIC	125	125	125	250
AI	0.55	0.66	0.76	0.57
A. flavus				
IZ	-	-	11.00±0.57	-
MIC	-	-	250	-
AI	-	-	0.43	-
A. niger				
IZ	10.00±0.00	11.66±0.66	11.00±0.57	10.00±0.00
MIC	500	500	250	250
AI	0.37	0.43	0.43	0.37
C. albicans				
IZ	11.33±0.34	10.66±0.66	14.33±0.33	10.00±0.00
MIC	125	250	125	250
AI	0.51	0.46	0.65	0.43
P. crysogenum				
IZ	10.66±0.67	10.66±0.67	15.00±0.00	10.00±0.00
MIC	500	250	62.5	1000
AI	0.50	0.50	0.71	0.47
T. rubrum				
IZ	10.66±0.66	11.00±0.00	15.00±0.00	10.00±0.00
MIC	250	125	62.5	500
AI	0.36	0.37	0.51	0.34

[a]Test samples 4 mg/well. Standard test drugs: Gentamycin for bacteria, ketonocozole for fungi (10 mcg disc^{-1}).[b] IZ: Inhibition zone (in mm) including the diameter of well (6 mm). MIC: Minimum inhibitory concentration in μg mL^{-1}. AI[d]: Activity index = Inhibition zone of the sample/Inhibition zone of the standard.

demonstrated 625.00±0.64 mg AAE (Table 2). In ferric thiocynate method, methanol extract exhibited maximum activity, i.e., % inhibition 90.74 (at 80 μg mL^{-1} concentration).

Table 2: Total phenolics, flavonoids and antioxidant activity by FRAP method

			Antioxidant activity in AAE[c]/g dw (Concentrations in μg mL^{-1})				
Type of extract	Total phenolics (GAE[a]/100 g)	Total flavonoids (in QE[b]/g dw)	1000 μg	500 μg	250 μg	125 μg	62.5 μg
Pet ether	52.33±0.87	53.33±1.33	255.00±2.8	110.00±5.00	100.00±2.89	86.66±3.33	71.66±3.33
Dichloromethane	53. 33±1.33	28.40±0.17	276.66±1.66	100.00±5.78	86.66±1.66	80.00±0.00	65.00±0.00
Ethyl acetate	66.66±0.64	29.61±0.43	333.33±1.66	273.33±3.33	205.00±0.00	103.33±1.66	90.00±2.89
Methanol	74.66±0.32	27.78±0.13	625.00±2.58	378.33±1.66	270.00±0.00	116.66±3.33	93.33±1.66
Ascorbic acid	-	-	1000	500	250	125	62.5

[a]GAE: Gallic acid equivalents, [b]QE: Quercetin equivalents, [c]AAE: Ascorbic acid equivalents

Table 3: Antioxidant activity by DPPH and ferrous ion chelating method

		% Inhibition (Concentrations in μg mL^{-1})									
		DPPH method					Ferrous ion chelating method				
Type of extract	IC_{50}	10	20	40	60	80	10	20	40	60	80
Pet. ether	27.5	18.70	19.75	21.43	26.50	34.71	72.04	72.23	73.18	77.12	77.36
Dichloromethane	25	16.70	21.43	24.21	25.46	26.50	74.32	75.08	75.58	75.79	78.20
Ethyl acetate	8.5	30.16	36.28	51.18	51.47	60.36	75.32	77.45	78.31	77.45	75.32
Methanol	6.5	75.33	76.66	78.35	81.82	84.43	76.83	77.55	77.83	79.30	90.74
Quercetin	4	62.42	80.58	93.38	93.82	94.71	73.25	76.77	83.87	85.53	86.03

% Inhibition = 1-(Absorbance of the sample/Absorbance of the control)×100

DISCUSSION

Most active antimicrobial activity was demonstrated by ethyl acetate extract having higher flavonoids content. Antibacterial activity of flavonoids has earlier been documented (Bylka *et al*., 2004; Cushnie and Lamb, 2005; Kosalec *et al*., 2008). In the present study antioxidant activity were better in methanol extract and demonstrated using DPPH method were DPPH accept free radical and become stable diamagnetic molecule that absorb maximum at 517 nm. Antioxidant molecule absorbs free radical and cause reduction in absorbance color changes purple to yellow (Chang *et al*., 2002), whereas, in ferrous ion chelating activity ferrozine quantitatively form complex with Fe^{+2}. In the presence of antioxidant compound this complex gets disrupted and formation of red color is imparted (Yamaguchi *et al*., 2000). Several diseases such as coronary heart disease, atherosclerosis, cancer, AIDS and ageing are caused due to oxidative damage (Finkel and Holbrook, 2000). Antioxidants are compounds that slow or prevent oxidation of biomolecules they prevent the formation of free radical as well as react with active electrons by their scavenging effects (Buyukokuroglu *et al*., 2001). Phenolics and flavonoids are the compounds which are widely distributed throughout the plant system demonstrated variable biological activities including antioxidant antiinflammatory; anticarcinogenic and antibacterial activities (Gryglewski *et al*., 1987).

CONCLUSION

The results further established the appreciable antioxidant and antibacterial activities which are attributed to the higher level of total phenolics, as present in methanolic extract. Similar observations have also been recorded by earlier workers (King *et al*., 1972; Dutra *et al*., 2008; Shukla *et al*., 2009).

ACKNOWLEDGMENTS

Authors are thankful to the Indian Council of Medical Research (ICMR), New Delhi, India, for partial financial support.

REFERENCES

Ali, M.S., I. Azhar, V.U. Ahmad and K. Usmanghani, 2001. Antimicrobial screening of Mimoceous plants. Pharm. Biol., 39: 43-46.

Boyanava, L., G. Gergova, R. Nikolov, S. Derejian and E. Lazarova *et al*., 2005. Activity of Bulgarian propalis against 94 *Helicobacter pylori* strains *in vitro* by agar well diffusion, agar dilution and disc diffusion methods. J. Med. Microbiol., 54: 481-483.

Buyukokuroglu, M.E., I. Gulcin, M. Oktay and O.I. Kufrevioglu, 2001. *In vitro* antioxidant properties of dantrolene sodium. Pharmacol. Res., 44: 491-495.

Bylka, W., I. Matlawska and N.A. Pilewski, 2004. Natural flavonoids as antimicrobial agents. J. Am. Nutraceut. Assoc., 7: 24-31.

Chang, L.W., W.J. Yen, S.C. Huang and P.D. Duh, 2002. Antioxidant activities of sesame coat. Food Chem., 78: 347-354.

Cushnie, T.P.T. and A.J. Lamb, 2005. Review antimicrobial activity of flavonoids. Int. J. Antimicrobial Agents, 26: 343-356.

Deshaprabhu, S.B., 1966. The Wealth of India-An Encyclopedia of India's Raw Material Resources. CSIR., New Delhi, India, pp: 291.

Dinis, T.C., V.M. Madeira and L.M. Almeida, 1994. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315: 161-169.

Duraipandiyan, V., M. Auyanar and S. Ignacimuthu, 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement. Altern. Med., 6: 35-35.

Dutra, R.C., M.N. Leite and N.R. Barbosa, 2008. Quantification of phenolic constituents and antioxidant activity of *Pterodon emarginatus* Vogel seeds. Int. J. Mol. Sci., 9: 606-614.

Finkel, T. and N.J. Holbrook, 2000. Oxidants, oxidative stress and the biology of aging. Nature, 408: 239-247.

Fogliano, V., V. Verde, G. Randazzo and X. Retieni, 1999. A method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. Agric. Food Chem., 47: 1035-1040.

Gryglewski, R.J., R. Korbut and J. Robak, 1987. On the mechanism of antithrombotic action of flavonoids. Biochem. Pharmacol., 36: 317-321.

Joshi, B.S. and V.N. Kamat, 1969. Identity of peltophorin with berginine. Naturwissenschaften, 56: 89-89.

King, A.D., H.G. Bayne, L. Jurd and C. Case, 1972. Antimicrobial properties of natural phenols and related compounds: Obtusastyrene and dihydro-obtusastyrene. Antimicrob. Agents Chemother., 1: 263-267.

Kosalec, I., S. Pepeljnjak, M. Bakmaz and S. Vladimir-kne, 2008. Flavonoid analysis and antimicrobial activity of commercially available propolis products. Pharm. Biol., 46: 634-638.

Maehly, A.C. and B. Chance, 1954. Methods of Biochemical Analysis. Vol. I, Wiley Interscience, New York, pp: 357.

Rahman, W., M. Ilyas and M.N. Hameed, 1969. Flower pigment flavonoids glycosides from *Peltophorum inermis*. J. Indian Chem. Soc., 46: 278-278.

Rahman, S.T., A. Shaukat, S. Ahmed and S.M.A. Waqar, 2007. Evaluation of solvent dependent antibacterial activity in flowers and shoots of *Peltophorum pterocarpum*. J. Chem. Soc. Pakistan, 29: 170-175.

Rao, D.S., 1965. Chemical investigation of *Peltophorum inermis*. Naturwissenschaften, 52: 262-262.

Satish, S., D.C. Mohana, M.P. Ranhavendra and K.A. Raveesha, 2007. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. J. Agric. Technol., 3: 109-119.

Satish, S., M.P. Raghavendra and K.A. Raveesha, 2008. Evaluation of the antibacterial potentials of some plants against human pathogenic bacteria. Adv. Biol. Res., 2: 44-48.

Sethuraman, M., N. Sulochana and L. Kameswaran, 1984. Antiinflammatory and antibacterial activity of *Peltophorum pterocarpum* flowers. Fitoterapia, 55: 177-179.

Shukla, S., A. Mehta, J. John, S. Singh, P. Mehta and S.P. Vyas, 2009. Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds. Food Chem. Toxicol., 47: 1848-1851.

Varshney, I.P. and N.K. Dubey, 1969. Chemical examination of flowers of *Peltophorum inermis* Roxb. J. Indian Chem. Soc., 46: 805-806.

Voravuthikunchai, S.P. and H. Mitchell, 2008. Inhibitory and killing activities of medicinal plants against multiple antibiotic-resistant *Helicobacter pylori*. J. Health Sci., 51: 81-88.

Yamaguchi, F., T. Ariga, Y. Yoshimura and H. Nakazawa, 2000. Antioxidative and antiglycation activity of garcinol from *Garcinia indica* fruit. J. Agric. Food Chem., 48: 180-185.

Yen, G.C. and H.Y. Chen, 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 43: 27-32.

Zhishen, J., T. Mengcheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.